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HPLC method for the determination of carboplatin and paclitaxel with cremophorEL in an amphiphilic polymer matrix☆

Anupama Mittal, Deepak Chitkara, Neeraj Kumar*

Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, SAS Nagar-160062, India

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Abstract

Simple and rapid reversed phase HPLC methods for individual as well as simultaneous analysis of paclitaxel and carboplatin with cremophorEL (CrEL) in an amphiphilic polymer matrix were developed. Different analytical performance parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines. All the analytical methods were developed by reverse phase HPLC on C-18 column with a mobile phase comprising of water–acetonitrile run on isocratic mode for the analysis of carboplatin and gradient mode for individual analysis of paclitaxel and for simultaneous analysis of the two drugs at a flow rate of 1 ml/min at 227 nm. The proposed methods for independent analysis of the drugs elute out carboplatin in 4.3 min and paclitaxel in 10.5 min while in simultaneous analysis carboplatin shows R_t at 4 min and paclitaxel at 18 min with a continuous run for 17 more minutes to elute out CrEL. These methods were found to be specific as none of the components of the media, i.e. polymer, CrEL and buffer interfered with the drug peaks. The linearity of the calibration curves for each analyte in the desired concentration range was found to be good ($r^2 > 0.9995$). The methods were accurate and precise with recoveries ranging from 98 to 101% for each drug and relative standard deviation (%RSD) <2%. Peaks corresponding to each of the drug showed positive value for the minimum peak purity index over the entire range of integrated chromatographic peak thus indicating the purity of the peaks. Stability analysis of the two drugs revealed that the drugs remain stable during the period of study.

Keywords: Reversed phase HPLC method; Paclitaxel; Carboplatin; CremophorEL

1. Introduction

A recent but rapidly evolving trend in cancer chemotherapy is the treatment of tumors by a combination of antineoplastic agents to achieve total tumor cell kill and to overcome the limited log kill of individual drugs. Synergistic combinations and rational sequences are devised by utilizing drugs which are effective when given alone, having different mechanisms of action and non-overlapping toxicities [1]. Paclitaxel and platinum analogs (cisplatin and carboplatin) is one such FDA approved combination administered sequentially for the treatment of different types of cancers; for example carboplatin in combination with paclitaxel is an approved therapy in patients with non small cell lung cancer (NSCLC), advanced and recurrent cervical carcinoma and gynecological cancers [2]. These drugs are conventionally administered by i.v. infusion in a sequential fashion, in which paclitaxel's clinical formulation consists of 1:1 (v/v) mixture of ethanol and CrEL which is diluted 5–20 folds in normal saline and dextrose isotonic solution prior to infusion. The formulation; however, suffers limitations in terms of stability, incompatibility with components of infusion sets and adverse undesired side effects of CrEL [3].

Our current interest lies in identifying the synergism of this combination when administered simultaneously via polymeric matrix, taking into consideration the dose reduction that would result particularly keeping in mind the high cost of paclitaxel and decreasing the population of residual resistant tumor cells and hence ensuring a more effective treatment. Additionally, the amphiphilic polymer, PLGA–PEG–PLGA (PLGA stands for poly(DL-lactic-co-glycolic acid) and PEG stands for poly(ethylene glycol) employed for designing this formulation) helps in solubilizing and stabilizing the drugs without the use of cremophorEL (CrEL) which is known to cause adverse reactions. Thus, it is necessary and justified to establish method for analysis of the two drugs (independently and simultaneously) in the presence of the polymer, buffer and CrEL (employed as

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* Corresponding author. Tel.: +91 172 2214683; fax: +91 172 2214692.
E-mail address: neeraj@niper.ac.in (N. Kumar).

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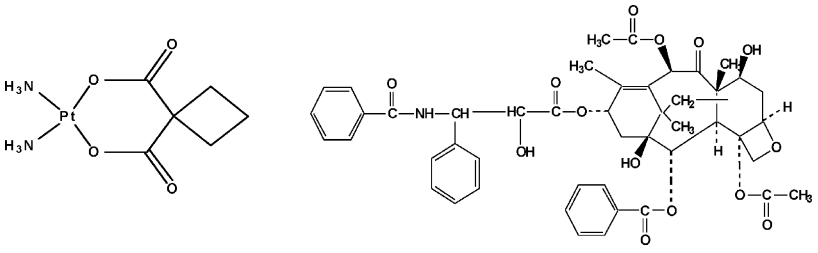
release media) using reverse phase HPLC in *in vitro* release study samples.

Carboplatin (*cis*-diamine-1,1-cyclobutanedicarboxylatoplatinum(II)) is a very hydrophilic molecule and has molecular weight of 371.3 Da. It is a cytotoxic drug and a second generation platinum-based antineoplastic agent which is a better substitute for cisplatin in combination regimens since the dose can be tailored to renal function and it has a more favorable non-hematological toxicity profile. It has a reported aqueous solubility of 14 mg/ml [4], one of its marketed formulations paraplatin (carboplatin aqueous solution) injection contains carboplatin 10 mg/ml [5]. The drug reacts with nucleophilic sites on DNA and proteins, forming both interstrand and intrastrand crosslinks [2]. It possesses broad antineoplastic activity. Development of a method by RP-HPLC was difficult owing to its extreme hydrophilicity; it has minimal *k* values on most ODS columns and is hence not retained on RP-based cartridges and it does not get partitioned extensively into water immiscible solvents [6,7]. Reported methods of analysis of platinum analogs are based upon atomic absorption (flame and nonflame) [8], inductively coupled plasma atomic emission (ICP-AE) [9], electroanalytical techniques [10], neutron activation analysis [11] and gas [12] and normal phase liquid chromatography [13].

Paclitaxel isolated from *Taxus brevifolia*, the Western Yew tree, on the contrary is a hydrophobic drug with a molecular weight of 853.90. It is a BCS class IV drug with extremely low solubility of 4 μg/ml [14]. Paclitaxel is an FDA approved drug for the treatment of patients with breast and ovarian cancers as well as lung and AIDS-related Kaposi's sarcoma (AIDS-KS).



(a) (b)

Structures of (a) Carboplatin (b) Paclitaxel

Different methods have been explored and employed for the determination of paclitaxel *viz.* capillary electrophoresis [15], LC–MS [16] and HPLC [17,18]. A critical literature survey reveals at this time that RP HPLC may be an overwhelming choice for its analysis. Although it shows good retention on many ODS columns; however, the main problem is with the low solubility of the drug. For this purpose, CrEL has been employed as a major component of paclitaxel formulations. CrEL solubilizes hydrophobic drugs by formation of a micelle, which creates a hydrophobic environment for the drug [19]. Nevertheless, it works as an excellent solubilizer for this drug; it certainly makes the analytical task of quantification a challenge. The chemical composition of CrEL has been studied, but not well characterized. It is composed of several different components with varying molecular weights, the major one being the hydrophobic glycerol–polyethylene glycol ricinoleate (80%) bonded to the hydrophilic polyethylene glycols and ethoxylated glycerol [19]. It elutes out in an ill defined pattern with reported run time of about 35 min [20]. Owing to its typical UV absorbance, it produces multiple interfering peaks when HPLC is used and thus becomes a major hurdle [19]. Other methods reported in literature for the analysis of paclitaxel in cremophor involves sample pretreatment that is also complicated, time consuming and expensive [20,21]. Moreover, liquid–liquid or solid phase extraction (SPE) often results in a loss of paclitaxel as seen in the case of SPE based sample treatment [19]. So as to avoid any such losses, which might yield erroneous results, the present study attempts to analyze paclitaxel individually and with carboplatin in the presence of CrEL, along with ensuring sufficient peak separation and purity.

Hydrophilicity of carboplatin and presence of CrEL in release media of paclitaxel release study samples create major hurdles in the individual analysis of these two drugs. Simultaneous determination is difficult owing to the diverse physicochemical properties of these two drugs, carboplatin being a highly soluble drug while paclitaxel being one of the least soluble molecules and presence of polyoxyl castor oil, cremophorEL that is added to create and maintain sink conditions for the release of the drugs particularly paclitaxel. Sink condition is the condition where drug is always below the saturated concentration. This is required for drug release purpose. Difference in the solubility of the two drugs demand a gradient mode wherein gradient changes from a weak solvent system (to elute carboplatin) to strong solvent system (to elute paclitaxel). The presence of CrEL (showing multiple interfering peaks) made the task of quantification even more intricate and harder. In this work a novel method for quantitative simultaneous determination of carboplatin and paclitaxel by RP-HPLC in the presence of cremophorEL has been reported. Analytical methods have been developed and validated as per ICH guidelines for analysis of each of the drug individually as well as in combination.

2. Experimental

2.1. Chemicals and reagents

Paclitaxel was obtained as a gift from Prof. Avi Domb, Hebrew University of Jerusalem, Israel and Carboplatin was generously gifted by Getwell Life Sciences (New Delhi, India). CrEL was purchased from Sigma–Aldrich (Germany)

and potassium dihydrogenorthophosphate (KH_2PO_4) from Spectrochem Pvt. Ltd. (Mumbai). Acetonitrile (HPLC grade) was obtained form J.T. Baker (USA). Water for chromatography and for preparation of stock and working solutions was obtained by reverse osmosis (ELGASTAT, UK). The triblock copolymer PLGA–PEG–PLGA was synthesized and characterized in our lab as per the reported method [22].

2.2. Preparation of stock solutions

Stock solutions of carboplatin (100 μg/ml) and paclitaxel (50 μg/ml) were prepared. Carboplatin and paclitaxel were weighed and dissolved in phosphate buffer (0.01 mM; pH 6.8) and in 10% (w/v) CrEL solution in phosphate buffer (0.01 mM; pH 6.8), respectively. The stock solutions were stored in amber colored volumetric flask at 4–6 °C. Working solutions for paclitexel were made by dilution of its stock solution with 10% (w/v) CrEL solution in phosphate buffer. Standard solution containing combination of carboplatin (50 μg/ml) and paclitaxel (50 μg/ml) was also prepared in the same medium and kept under same storage conditions. To establish specificity in polymer matrix, a 30% (w/w) polymer solution was also prepared by adding PLGA–PEG–PLGA to water and sonicated for an hour; this produces degradation products, oligomers and monomers. This solution was used to spike the samples of carboplatin, paclitaxel and their combination.

2.3. Equipment and HPLC conditions

Shimadzu HPLC system has been employed to develop the methods. System consists of a quaternary pump (LC-10ATVP), an autoinjector (SIL-10AD VP), column oven (CTO-10ASVP), degasser unit (DGU-14AM) and system controller (SCL-10AVP). The system was equipped with a PDA detector (SPD-M10A VP). Chromatographic separations were carried out using the Inertsil® ODS-3V column purchased form GI Sciences Inc., 5 μm, 25 cm × 4.6 mm and guard column employed was Symmetry® C_{18}. Mobile phase consisted of a mixture of acetonitrile and purified water. Mobile phases were filtered through 0.22 μm nylon filter and degassed in ultrasonic bath sonicator for 60 min before running the experiment. Table 1 shows the mobile phase compositions employed for analysis of both the drugs individually as well as in combination. Flow rate was kept at 1 ml/min and system was maintained at 35 °C, the detection was carried out at λ = 227 nm. Injection volume was 20 μl. Data was acquired and processed by Class-VP software (Shimadzu, Japan). Fig. 1 shows chromatograms obtained by each analytical method (Table 1).

2.4. Solution state stability testing at working pH

Stability testing was carried out to evaluate the stability and extent of degradation of the stock solution containing both the drugs in the presence of 10% (w/v) CrEL solution in phosphate buffer (0.01 mM; pH 6.8). Fresh stock solution of the combination containing carboplatin (50 μg/ml) and paclitaxel (50 μg/ml) was prepared, working solutions at three concentration levels were made from this standard solution and kept at 4–6 °C. Sampling was done at regular time intervals for a period of 7 days in triplicate. Each sample was run in HPLC after filtering through 0.22 μm filter. The peak areas of the individual drugs were compared at different time points to determine the stability as a function of time.

2.5. Peak purity assessment

Peak purity was assessed using class VP software for Shimadzu HPLC system based on the degree of similarity of UV spectra across the peak in the range of 190–800 nm. Peak purity evaluation was performed with the objective of obtaining additional supportive information during selection of appropriate analytical conditions that allowed specific determination of both carboplatin and paclitaxel. The peak was classified as pure if the peak purity index was greater than the single point threshold resulting in a positive value of minimum peak purity index.

2.6. Validation of analytical method

Each of the developed method was validated as per ICH guidelines for linearity, accuracy and precision, and specificity [23,24]. LOD and LOQ were determined using serial dilution method.

Table 1
Chromatographic conditions for individual and simultaneous analysis of carboplatin and paclitaxel

Drugs	Mode	Time (minutes)	ACN:water
Carboplatin	Isocratic made	5	10:90
	Equilibration	0.01	50:50
	Linear gradient	15	100:0
Paclitaxel	Isocratic mode	23	100:0
	Re-equilibration	26	50:50
		31	50:50
	Equilibration	0.01	3:97
	Linear gradient	17	100:0
Simultaneous analysis (carboplatin and paclitaxel)	Isocratic mode	25	100:0
	Linear gradient	30	3:97
	Re-equilibration	35	3:97

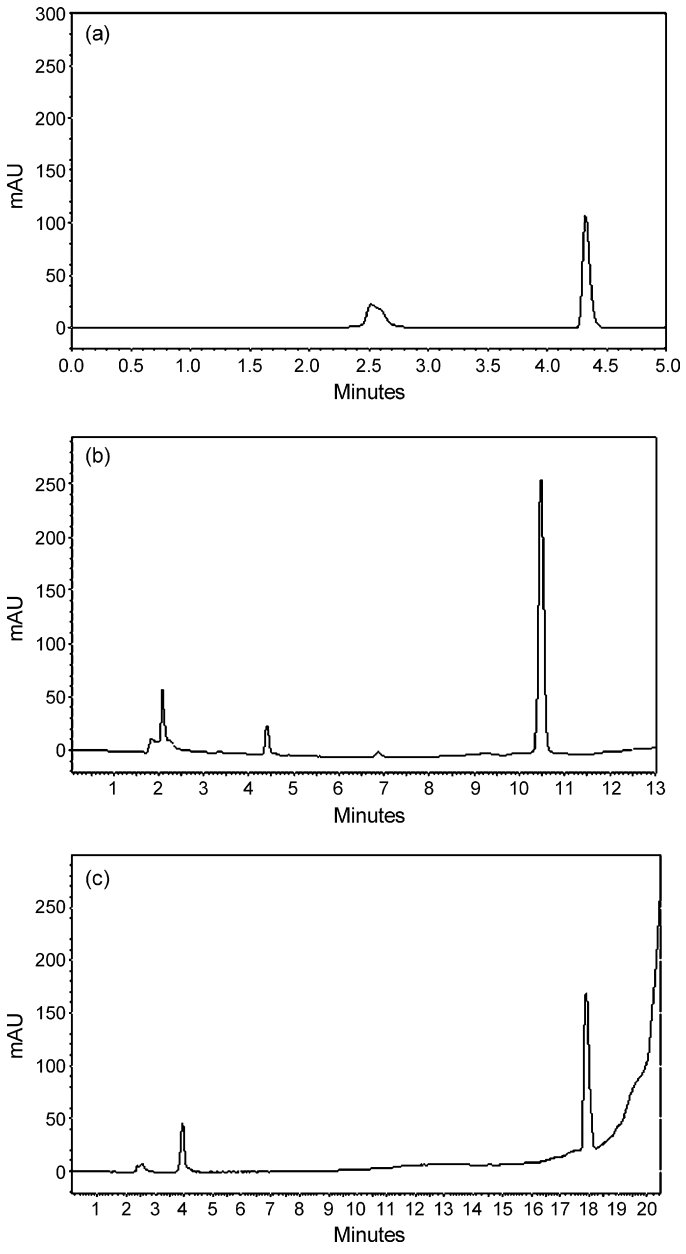


Fig. 1. Figure illustrating the chromatograms obtained by each of the three methods: (a) chromatogram showing well separated peak of carboplatin (R_t = 4.3 min) from that of the polymer and buffer (both give a combined distorted peak seen at 2.3 min) in individual analysis of carboplatin; (b) chromatogram showing well separated peak of paclitaxel (R_t = 10.5 min) from that of the polymer, buffer (solvent front seen at 2.3 min) and cremophor (minor peak at 4.3 min) in individual analysis of paclitaxel; (c) representative chromatogram of carboplatin (at 3.8 min) and paclitaxel (at 18 min) obtained by the proposed method. Disturbance at 2.3 min corresponds to that of buffer and polymer.

2.6.1. Linearity

The linearity of the methods used for carboplatin and paclitaxel analysis was evaluated from the standard curve of detector response (peak area) against analyte concentration. The concentration range was selected on the basis of anticipated drug concentration in the release study samples and six point calibration curves were generated on 3 consecutive days with standard working solutions of carboplatin, paclitaxel and their combination. The solutions were injected in triplicate into the HPLC column. Linearity of the analytical procedure was evaluated by plotting detector response (peak area) against analyte concentration. Linear regression analysis was applied to calculate the slope, intercept and linear correlation coefficient (r^2).

2.6.2. Accuracy and precision

Accuracy and precision of the analytical method was determined by analyzing quality control samples (QC) at three different concentrations within the calibration range in triplicate (n = 3). QC standards were prepared in the same media and are dilutions from weightings independent from those used for preparation of calibration curves.

The precision (%RSD) of the analytical procedure was evaluated by determining the intra- and inter-day coefficient of variation and reported as %RSD for a statistically significant number of replicate measurements. The intra-day precision of the selected method was estimated by the analysis of three different concentrations of the drug in triplicate and three times on the same day. The interday precision was assessed by analyzing samples in the same way as for intraday precision assay, and was repeated for three consecutive days.

2.6.3. Specificity

The specificity is the ability of the analytical method to measure accurately and specifically the analyte of interest in the presence of other components that might be expected to be present in the sample matrix. Specificity of analytical method was evaluated for both the drugs individually and in combination with the polymer matrix. The method specificity was assessed by comparing the chromatograms obtained from drugs alone and of those obtained from the spiked samples and through the peak purity curves.

2.6.4. Quantitation limits

LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte while LOQ is the lowest amount of the analyte in a sample, which could be quantitatively determined with suitable precision and accuracy. LOQ was assessed by standard deviation of the response and the slope method. Slope S was calculated based upon the calibration curve of the analyte and the standard deviation was estimated by running five blank samples while LOD was taken as one-third of LOQ in the case of analysis of carboplatin (independent analysis) while for paclitaxel (independent analysis) and for their simultaneous analysis, LOQ and LOD were estimated by serial dilution method since due to the ill defined peaks and humps produced by cremophorEL, standard deviation of the blank was not possible to be estimated.

3. Results and discussion

3.1. Chromatographic separation

The mobile phase composition was designed to retain carboplatin on the ODS column so that its peak was not interfered

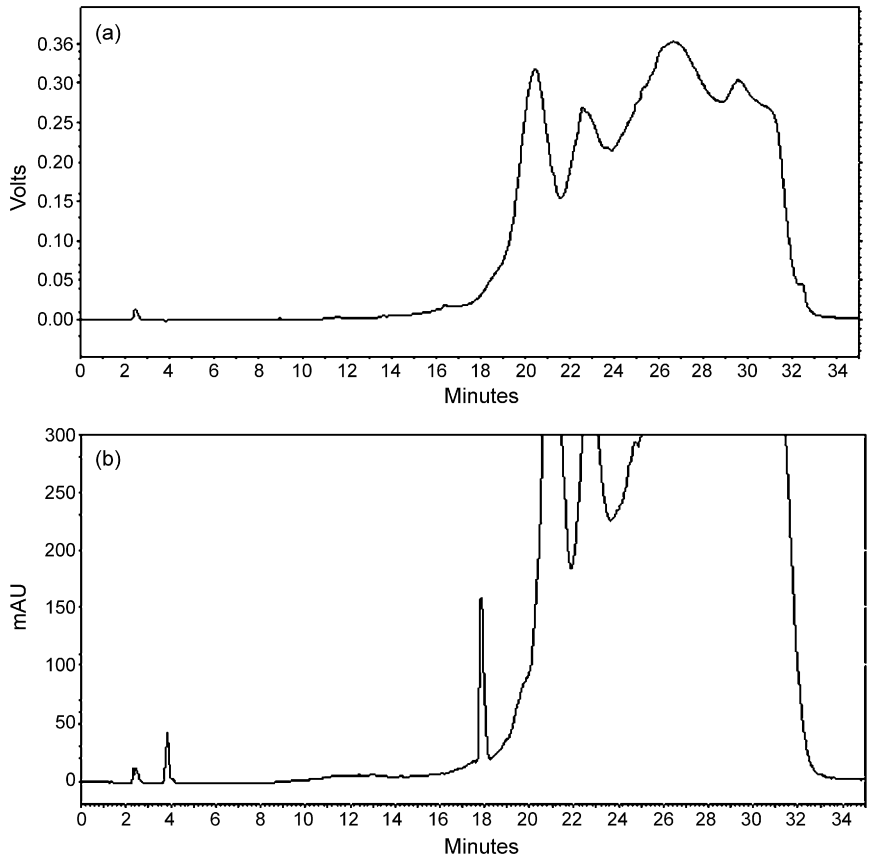


Fig. 2. (a) Chromatogram of the blank (polymer and 10% (w/v) cremophorEL in phosphate buffer) without the drugs. No peak is seen at the retention times of either carboplatin (3.8 min) or of paclitaxel (18 min). (b) Complete chromatogram showing the peaks of carboplatin at 3.8 min and that of paclitaxel at 18 min, total run time was extended to 35 min to elute out CrEL from the column. As seen in the figure above, CrEL gives an irregular pattern and has longer elution time.

by polymer and phosphate buffer which was seen to elute at ~2.3 min. Different mobile phase solvents were screened at various compositions and the final working mobile phases have been listed in Table 1. Ratio of ACN:water was optimized at 10:90 wherein carboplatin showed a retention time of 4.3 min and was well separated from the solvent front of phosphate buffer in which the working solutions were prepared, the spiked samples also showed sufficient purity and specificity. In case of paclitaxel, it was seen that cremophor eluted-out in an ill defined pattern and gave both minor peaks and broad humps all throughout the chromatogram. Peak of paclitaxel was needed to be separated from these interferences. To achieve this end, both linear gradient and isocratic mode were employed in the gradient program and suitable gradient mode was finally optimized (Table 1). Paclitaxel was seen to elute out at $R_t = 18$ min. Nevertheless, the drug got eluted in 18 min; the run was further continued for 17 min to ensure the complete removal of cremophor from the column and to re-equilibrate the system to initial conditions. Fig. 2(a) and (b) illustrate the chromatogram of blank (without the drugs) and the complete chromatogram generated over 35 min showing peaks of both the drugs along with the excipients, respectively. The gradient designed for the simultaneous analysis of the two drugs was also optimized on similar basis.

3.2. Stability of stock solutions

Table 2 shows stability data of the stock solution containing both the drugs. The stock solution of the combination was found to be stable for 1 week as %recovery was within the statistical

Table 2
Stability of the stock solution containing carboplatin and paclitaxel in the presence of 10% (w/v) cremophorEL in phosphate buffer over a period of 7 days (100 Mm; pH 6.8)

Drug	Concentration (μg/ml)	Day 3	Day 7
Carboplatin	50	97.96 ± 0.26 (0.27)	98.42 ± 0.09 (0.9)
	40	99.75 ± 0.20 (0.20)	100.18 ± 2.22 (2.2)
	30	99.67 ± 0.61 (0.61)	99.82 ± 0.57 (0.57)
Paclitaxel	50	100.76 ± 0.20 (0.20)	101.31 ± 0.11 (0.10)
	40	99.98 ± 0.41 (0.41)	98.46 ± 0.10 (0.10)
	30	98.85 ± 0.83 (0.84)	97.21 ± 0.36 (0.37)

Chromatograms obtained by running three concentrations on 3rd and 7th day from the preparation stock solution have been compared with those obtained initially. Values given under day 3 and day 7 denote peak area ± SD (%RSD) calculated with respect to the average peak area of the respective concentrations as obtained initially. Recovery and % RSD are seen to be within statistical limits, hence, the solutions remain stable over a period of 7 days under 4–6 °C.

Table 3
Validation parameters of the HPLC method of carboplatin alone, paclitaxel alone and carboplatin and paclitaxel in combination

Parameter	Carboplatin alone	Paclitaxel alone	Simultaneous analysis	
			Carboplatin	Paclitaxel
Analytical wavelength	227 nm	227 nm	227 nm	227 nm
Linearity (μg/ml)	5–100	2–50	2–50	2–50
Slope	9460.1 ± 54.90	38496 ± 526.04	8131.2 ± 55.84	38808.67 ± 682.547
% RSD of slope	0.58%	1.37%	0.69%	1.75%
Intercept	8426.47 ± 637.79	45999 ± 33451.42	2601 ± 1259.948	−34516.9 ± 30663.8
Correlation coefficient (R^2)	0.9994 ± 0.07	0.9999 ± 0.01	0.9998 ± 0.017	0.9997 ± 0.026
LOD (μg/ml)	1.06	0.17	1.0	0.2
LOQ (μg/ml)	3.2	0.5	3.0	0.6

Each standard curve was generated in triplicate on 3 consecutive days distributed evenly across the linearity range. Values are reported as mean ± SD of three calibration curves.

Table 4
Results of accuracy and precision studies of carboplatin alone, paclitaxel alone and carboplatin and paclitaxel in the presence of excipients

	Drug concentration (μg/ml)					
	Inter-day			Intra-day		
Carboplatin	80	60	40	80	60	40
Precision (%RSD)	1.42 ± 1.13	0.43 ± 0.31	1.02 ± 0.40	0.97 ± 0.77	2.35 ± 1.44	0.89 ± 0.35
Accuracy (% Recovery)	100.0 ± 1.42	99.99 ± 0.52	99.99 ± 1.02	100.00 ± 0.98	100.00 ± 2.35	100.00 ± 0.89
Paclitaxel	45	30	15	45	30	15
Precision (%RSD)	0.90 ± 0.41	0.77 ± 0.23	1.53 ± 0.24	1.06 ± 0.49	1.77 ± 0.53	1.11 ± 0.17
Accuracy (% Recovery)	100.0 ± 1.38	99.99 ± 0.66	99.97 ± 1.52	99.63 ± 1.22	99.74 ± 2.42	100.63 ± 0.41
Simultaneous analysis						
Carboplatin	35	25	15	35	25	15
Precision (%RSD)	1.47 ± 0.51	1.26 ± 0.31	1.27 ± 0.19	1.07 ± 0.37	1.56 ± 0.38	1.38 ± 0.19
Accuracy (%Recovery)	98.78 ± 1.47	99.46 ± 1.26	100.62 ± 1.27	97.91 ± 1.07	98.81 ± 1.56	97.56 ± 1.31
Paclitaxel	35	25	15	35	25	15
Precision (%RSD)	1.08 ± 0.38	0.85 ± 0.21	0.82 ± 0.123	1.17 ± 0.40	0.84 ± 0.21	1.18 ± 0.17
Accuracy (%recovery)	101.17 ± 1.08	100.02 ± 0.85	99.92 ± 0.82	98.39 ± 1.17	98.6 ± 0.84	96.61 ± 1.82

Accuracy and precision were determined with QC samples. Triplicate samples were analyzed on three consecutive days. For intraday determinations, three standard curves were prepared on the same day. For interday determinations, three standard curves were generated on three consecutive days. The accuracy is represented by %recovery (mean ± SD) and precision by %RSD.

limits. Further, no appreciable change was observed in the measured concentration of the drugs in the presence of excipients, i.e. polymer, CrEL and buffer during the period (Table 2).

3.3. Validation of the method

The methods developed for analysis of carboplatin and paclitaxel individually and simultaneously were validated for linearity, accuracy, precision, specificity, and quantification limits as per ICH guidelines. Linear regression analysis confirms that the r^2 values for both the drugs were found to be >0.9995, confirming the linear relationship between the concentration of the drug and area under the curve. Validation parameters have been highlighted in Table 3 for carboplatin and paclitaxel individually and for their simultaneous analysis. Purity of the peaks corresponding to the drugs in each of the methods was also established as an additional proof of specificity.

Accuracy and precision data show that the recoveries ranged from 98 to 101% for carboplatin as well as for paclitaxel. Both intra- and inter-day precision (%R.S.D.) of QC standards were less than 2% over the selected range for both the drugs (see Table 4). The calculated LOD and LOQ concentrations confirmed that the methods were sufficiently sensitive.

Specificity evaluation was carried out by analyzing carboplatin and paclitaxel separately. It was observed that the peak of each of the drugs was well separated and not being interfered by polymer, buffer or cremophorEL. Recovery of both drugs from solutions prepared with CrEL and buffer in water

Table 5
Results of specificity studies

Drug	Actual concentration (μg/ml)	Calculated concentration (μg/ml)	%Recovery
Carboplatin	50	50.62 ± 0.85 (1.67)	101.25
	30	29.66 ± 0.37 (1.24)	98.88
	10	9.90 ± 0.08 (0.079)	99.03
Paclitaxel	45	44.63 ± 0.26 (0.58)	99.18
	30	30.26 ± 0.25 (0.83)	100.87
	15	14.96 ± 0.51 (3.40)	99.76

Spiked samples were analyzed at three concentrations in triplicate on 3 consecutive days, results shown indicate the concentration ± SD. (%RSD) as calculated from the standard curves generated on the respective days.

was accessed at three concentration levels in triplicate. Table 5 shows the recoveries of both carboplatin and paclitaxel were within statistical limits. Further, peaks corresponding to each of the drugs obtained by the proposed method were seen to be pure (Fig. 3). Thus, the method was confirmed to be specific for each of the two drugs individually as well as in combination in the presence of excipients like polymeric matrix, buffer and CrEL. Hence, the methods were suitably employed for quanti-

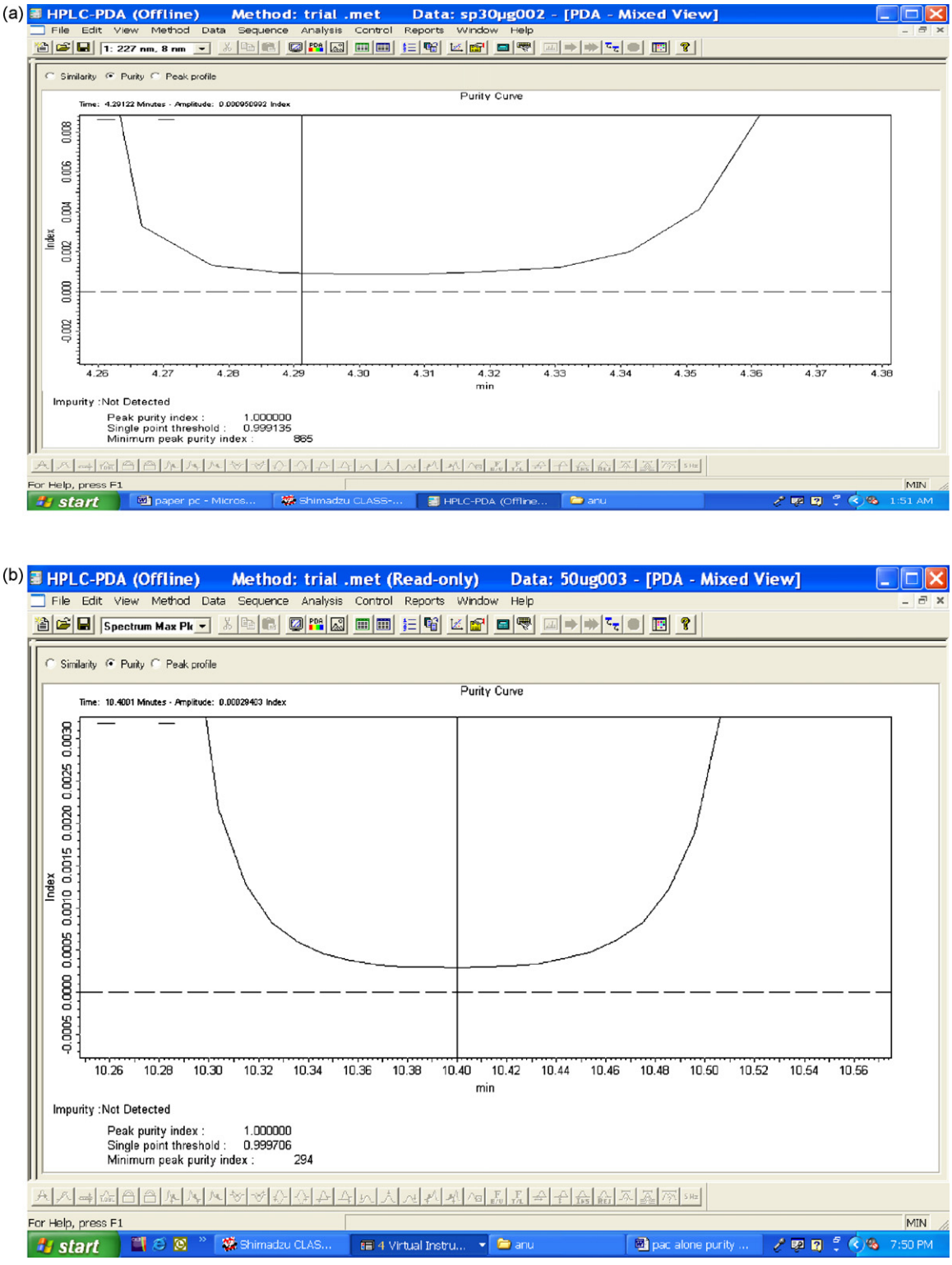


Fig. 3. Representative peak purity curves of: (a) carboplatin in individual analysis of carboplatin; (b) paclitaxel in individual analysis of paclitaxel; (c) carboplatin in simultaneous analysis; (d) paclitaxel in simultaneous analysis. Each of the peaks has a positive value of minimum peak purity index confirming the purity of the peaks and specificity of each of the methods developed in the presence of excipients like polymer matrix, buffer and cremophor.

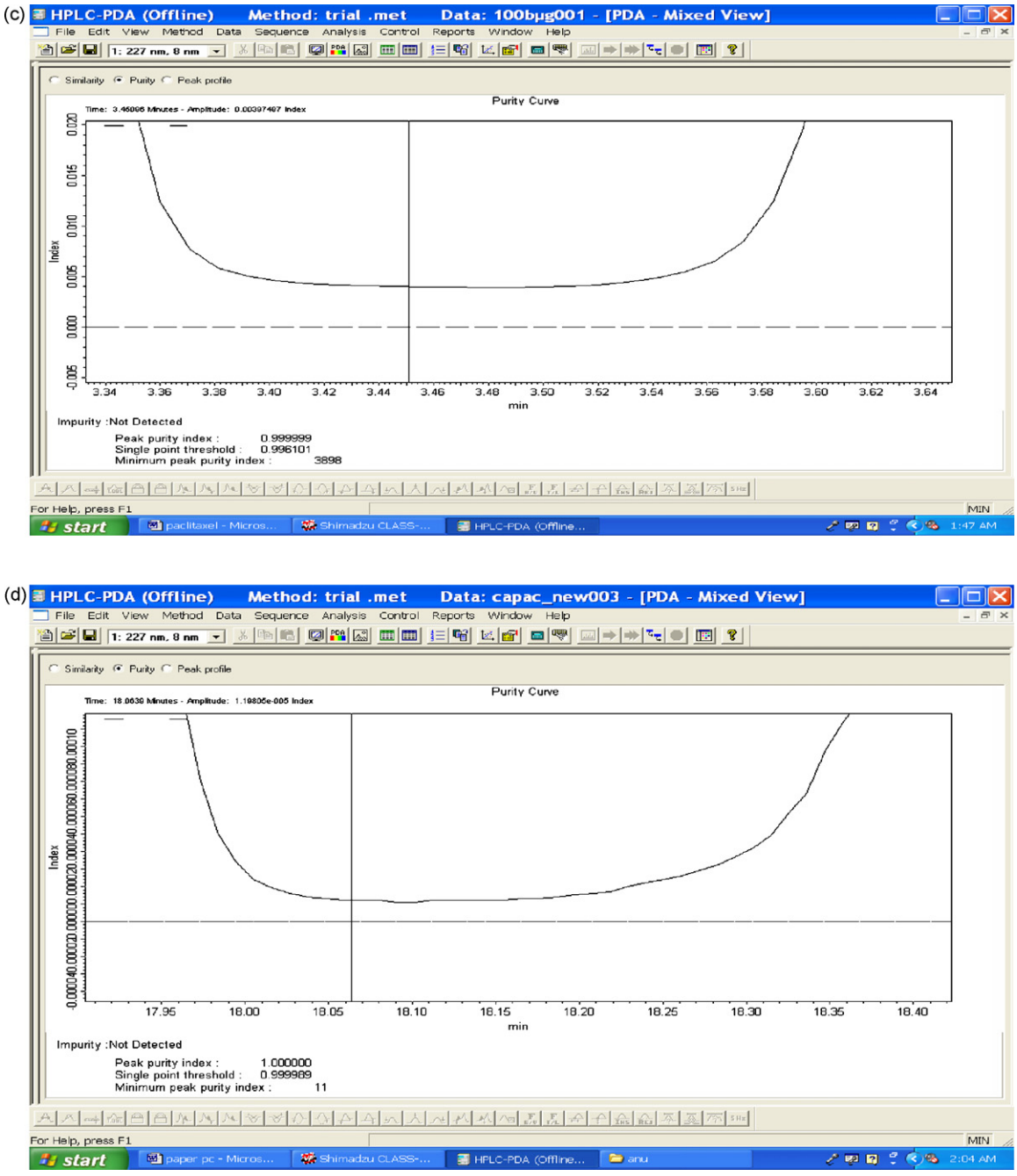


Fig. 3. (*Continued*).

tative analysis of carboplatin, paclitaxel and their combination in the presence of excipients in the *in vitro* release studies.

4. Conclusions

The HPLC method was developed for individual as well as simultaneous determination of two drugs namely carboplatin and paclitaxel in the presence of polymer matrix and was found to be suitable for the analysis. The proposed methods are simple, rapid and reliable enough to be employed for analysis of the two drugs individually and simultaneously employing the same mobile phase solvents, water and acetonitrile in both the methods with modification in their composition. Use of purified water in the method has an edge above the use of buffer since buffer puts additional pressure on the column and requires more of column washing. Run time for carboplatin (4.3 min) ensures its rapid estimation without any interference from either buffer or the polymer. Paclitaxel elutes out in 10.5 min with total run time of 31 min, which seems to be reasonable as the reported run time for paclitaxel in the presence of CrEL is 35 min. Run time of 35 min seems to be conducive for simultaneous analysis of the two drugs considering the difference in polarity of the two drugs and presence of excipients. Gradient program has been designed such that step gradient is not involved since step gradient is

known to give sudden shock and stress to the column diminishing its life. Validation report confirms that the method has good linearity, accuracy, precision, adequate specificity and purity.

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